# Nonclinical Development of Biotechnology-Derived Products and Small Molecules: What are the Differences?

Melanie T. Hartsough, Ph.D.
Pharmacologist
CDER, FDA

# Outline

- Inherent differences between small molecules and biotechnology-derived products: pharmacology/toxicology perspective
- Nonclinical development plans
  - Studies needed to initiate Phase 1 trials
  - Studies needed for licensure (NDA or BLA)
- Guidances
- Take home points

# Definitions for this presentation Biologics vs. Small Molecules

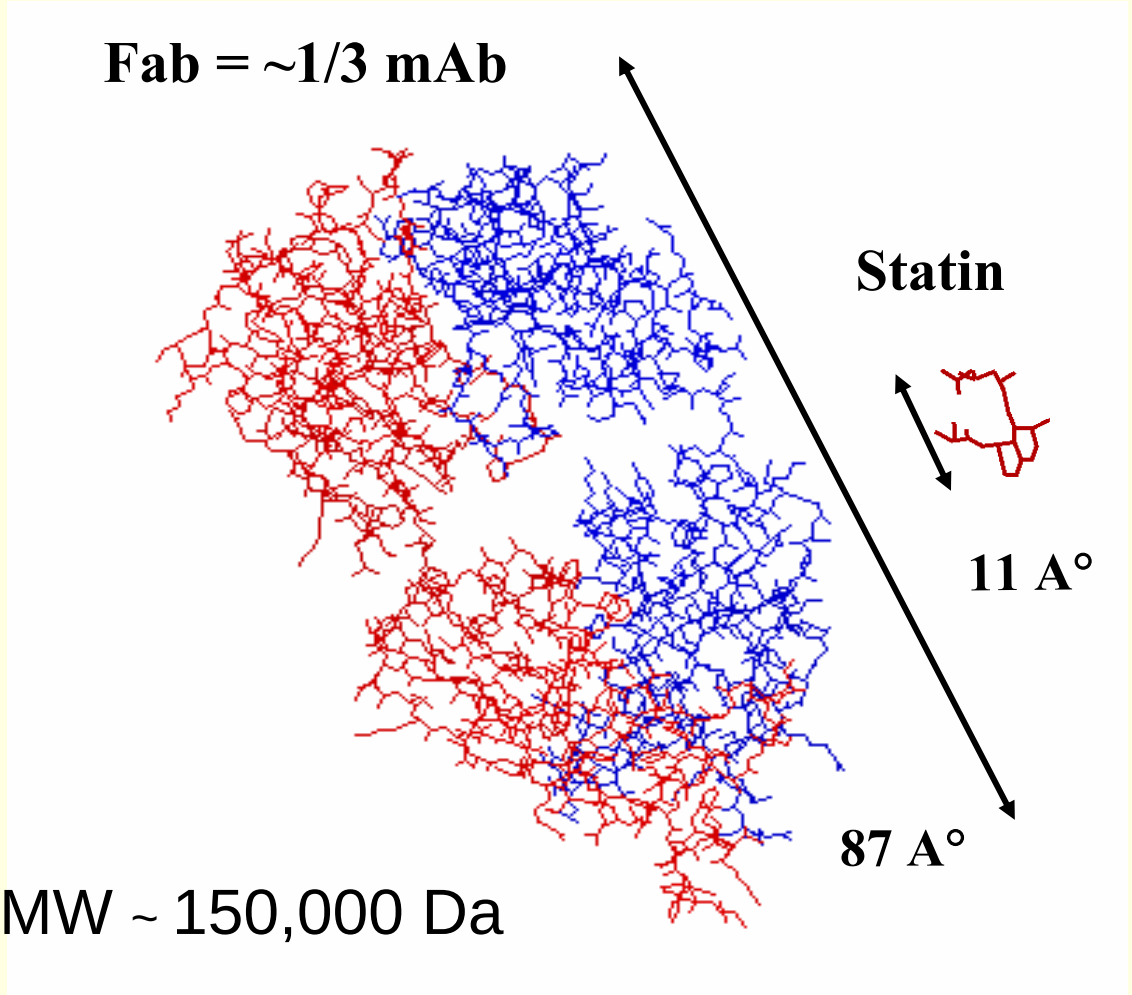
- Biologics = proteins such as monoclonal antibodies, cytokines, growth factors, enzymes, thrombolytics, etc
- Small molecules= typical low molecular weight molecules

# Differences Between Biologics and Small Molecules

- **Biologics**
  - From living cells
  - High molecular weight
  - Highly targeted
  - Responsive (relevant) and nonresponsive species
  - Immunogenicity
  - Proteolytic degradation
  - Exaggerated pharmacology
- **Small Molecules**
  - Chemically synthesized
  - Low molecular weight
  - Less targeted
  - Generally active in many species
  - Generally no immunogenicity
  - Metabolism
  - Toxicity from parent or metabolite

# Structure
# Biologics vs. Small Molecules

- 1° structure
- Higher order structure
- Post translational modification
- Heterogeneity




Statin MW ~400 Da
Monoclonal Antibody MW ~ 150,000 Da

# Consequence of Manufacturing from Living Cells-- Biologics

- Complexity of protein structure results in microheterogeneity of final product
  - Glycosylation, deamidation, oxidation, disulfide bonds, free thiols, clipping, aggregates
- Manufacturing of biologics evolves with product development (i.e., frequent changes, scale-ups, etc)
- Since manufacturing changes may alter product, comparability assessments are frequently performed
- Comparability assessment ensures that the manufacturing changes have not affected the safety, identity, purity or efficacy, including immunogenicity, of the product
- Analytical and functional assays performed (quality attributes/ CMC issues)

# Consequence of Manufacturing from Living Cells-- Biologics

- Product used in nonclinical studies not required to be “identical” to that going into the clinic, but does have to be “comparable” in order to extrapolate the preclinical safety data to the clinical scenario
- If product comparability cannot be established with quality assessments, preclinical studies may be required
  - PK assessments
  - PD assessments
  - Toxicology studies

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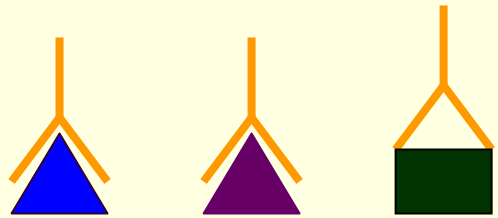
# What is a Pharmacologically Relevant Species?

- Relevant species is one in which the biological product is <u>active</u> due to the presence of a receptor/epitope
- Binding specificity and affinity of biologics often limit species cross-reactivity



**Anti-human TNF-α antibody**

▲ = **Human TNF-α**

▲ = **Cynomolgus TNF-α**

■ = **Murine TNF-α**

# Relevant Species
# Biologics vs. Small Molecules

- The need to conduct toxicology studies for biologics in a pharmacologically relevant model can result in toxicology studies being conducted in a single species
  - Studies in non-relevant models can be misleading and are discouraged
- In contrast, toxicology studies for small molecules are performed in two species (one rodent and one nonrodent)

# Relevant Species Biologics

- Relevant species for a biologic is frequently limited to a nonhuman primate
  - Cynomolgus monkey most commonly used
  - Very heterogeneous population compared to rat and dog
  - More difficult to obtain than rat and dog
  - Limitations for reproductive toxicology and carcinogenicity studies

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# Immunogenicity
# Biologics vs. Small Molecules

- Immunogenicity = immune reaction against product, resulting in production of anti-product antibodies
  - Biologics
    - Foreign proteins evoke immune responses
    - Testing for anti-product antibodies required
  - Small Molecules
    - Immune response not mounted against small molecules
    - Testing for anti-product antibodies not necessary

# Immunogenicity Biologics

- Anti-product antibodies can affect toxicology studies
  - Decrease activity- neutralize the product's activity or increase clearance
  - Sustain activity- decrease clearance
  - Cause adverse effects not directly related to the product (e.g., immune complex deposition)
  - Cross-react with endogenous proteins
  - Cause no effect

# Immunogenicity Biologics

- Included as an endpoint in toxicology studies
  - Aid in the interpretation of toxicology study
- Presence of anti-product antibodies is not a reason to terminate study early
- Studies are terminated when anti-product antibodies cause loss of product exposure (i.e., neutralize activity or increase elimination)
- Immunogenicity in animals not necessarily predictive of humans (e.g.,may be foreign in animals but not in humans)

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# Clearance – P450 Metabolism Small Molecules

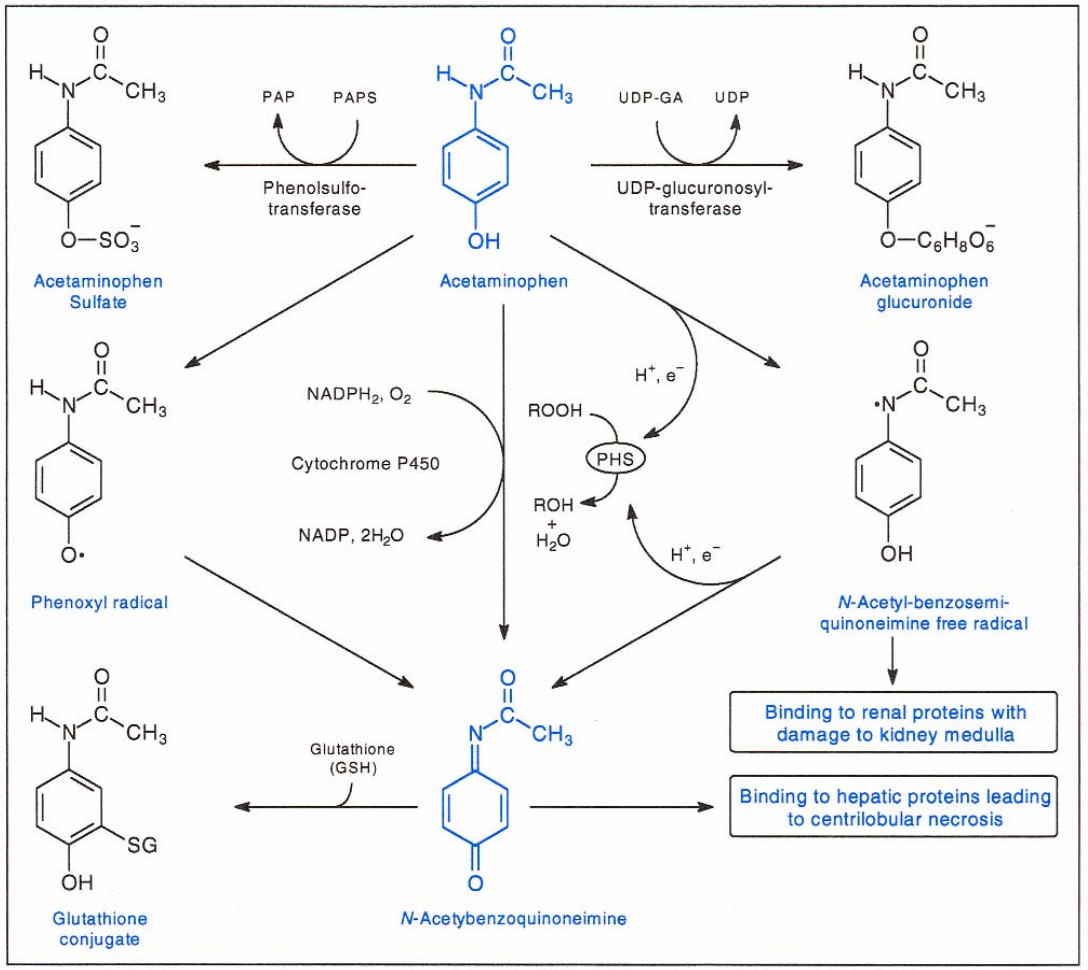


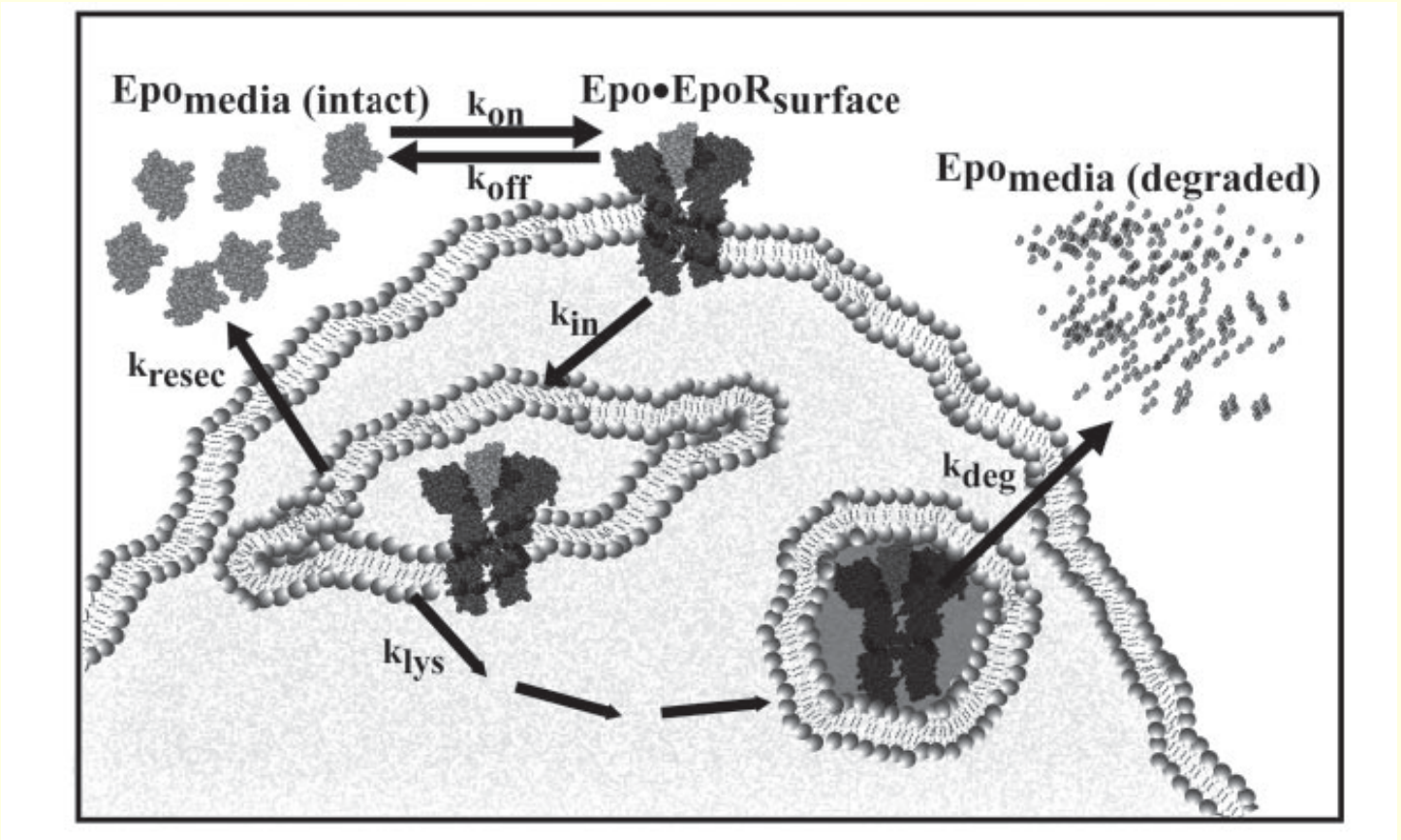

*Figure 6-28. Activation of acetaminophen by cytochrome P450, leading to hepatotoxicity, and by prostaglandin H synthase (PHS), leading to nephrotoxicity.*

Acetaminophen metabolism

(Casarett and Doull, 2001)

# Clearance - Receptor Internalization and Degradation-- Biologics

Erythropoietin Clearance




Gross and Lodish, JBC 281: 2023-2032, 2006

# How Does the Nonclinical Development of Biologics and Small Molecules Differ?

# Types of Nonclinical Studies

- Pharmacodynamics
- Tissue Cross-reactivity
- Pharmaco/Toxicokinetics
- Local Tolerance
- General Toxicology
- Safety Pharmacology
- Genotoxicity
- Reproductive Toxicology
- Carcinogenicity
- Immunotoxicity
- QT Prolongation

# Good Laboratory Practices (GLPs) 21 CFR Part 58

- GLPs are a set of organizational requirements to assure generation of high quality, reliable safety data
  - Analysis of test article, testing of dosing solutions, qualifications of study personnel, record keeping, etc.
- GLP requirements are the same for biologics and small molecules
  - All toxicology studies (i.e., safety pharmacology, general tox, genotox, repro tox, carcino) expected to be GLP compliant

# Preclinical Studies Needed for a Phase 1 Study

| Study | Biologics | Small Molecules |
|---|---|---|
| **Pharmacology** | Yes | Yes |
| **Safety Pharmacology** | Maybe<br>(usually incorporated in general toxicology study) | Yes<br>(usually standard models) |
| **Pharmaco/Toxicokinetics** | Yes<br>(1 species acceptable) | Yes<br>(2 species) |
| **Toxicology (acute/repeat dose)** | Yes<br>(1 species acceptable) | Yes<br>(2 species) |
| **Genotoxicity** | Generally Not Required | Yes |

ICHM3: Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals

# Preclinical Studies Needed for a Phase 1 Study

| Study | Biologics | Small Molecules |
|---|---|---|
| **Tissue cross-reactivity** | Yes<br>(monoclonal antibodies) | Not Required |
| **Local Tolerance** | Yes<br>(incorporated in toxicology study) | Yes |

# Safety Pharmacology Studies

- Safety pharmacology studies asses the potential undesirable pharmacodynamic effects of a substance on physiological functions
- Core battery: CNS, cardiovascular and respiratory systems
- For highly targeted biologics, safety pharmacology endpoints may be included in toxicology or pharmacodynamic studies

ICH S7A: Safety Pharmacology Studies for Human Pharmaceuticals

# Pharmacokinetics
## Biologics vs. Small Molecules

- Absorption
  - Same principles apply to biologics and small molecules
- Distribution
  - Biologics: high molecular weight proteins mainly stay within the vascular space for a significant period of time
    - Do not diffuse

# Pharmacokinetics
# Biologics vs. Small Molecules

- Metabolism
  - Biologics
    - Proteolytic degradation, no metabolites
    - Amino acids recycled- used for energy or new protein synthesis
  - Small molecules
    - P450 metabolism
    - Active metabolites may be formed

# Pharmacokinetics
# Biologics vs. Small Molecules

- Elimination
  - Biologics
    - Major form of elimination from circulation is binding to receptor/antigen and internalization
    - Anti-product antibodies and receptor saturation can alter product clearance and half-life
    - Mass balance studies are noninformative
  - Small Molecules
    - Major form of elimination may be excretion in urine, billiary or renal secretion, metabolism
    - Mass balance studies useful

# Tissue Cross-reactivity Studies Biologics- Monoclonal Antibodies

- Assess ability of monoclonal antibodies to bind to target and non-target tissues using immunohistochemistry
- Conducted using human tissues obtained at autopsy or during surgery
- Animal tissues are stained to verify model selection for toxicology studies

# Toxicology studies
# Biologics vs. Small Molecules

- Biologics
  - Toxicities usually exaggerated pharmacology
    - Mechanism of action is important in determining adverse events
  - Highest dose tested is..
    - A scientifically reasonable multiple of the highest projected clinical dose
    - Maximum feasible dose
    - A dose reflective of a pharmacodynamic marker (e.g., saturation of antigen)
- Small Molecules
  - Toxicities may be from parent compound or metabolite
  - Highest dose tested is…
    - Maximum tolerated dose

# Genotoxicity
# Biologics vs. Small Molecules

- Assays designed to detect substances that directly interact with DNA and induce gene mutations, chromosome aberrations and/or DNA damage
- Small molecules
  - Standard 3 assays performed
- Biologics
  - Genotoxicity assays typically not performed
    - Not expected that proteins would interact with DNA
    - Manufacturing involves physical separation as opposed to the introduction of organic chemicals
  - Presence of organic impurities or organic linkers might warrant genotoxicity testing

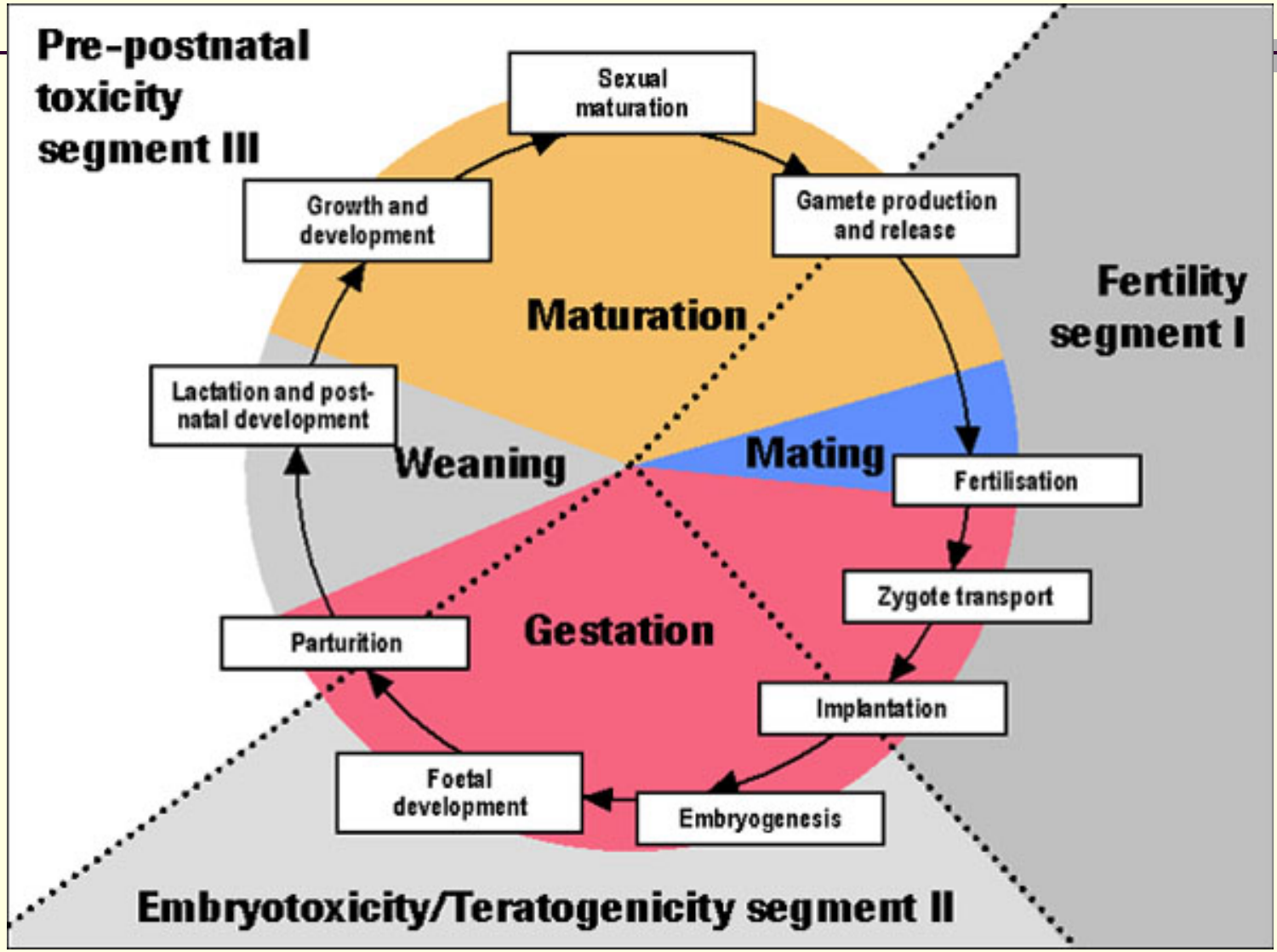
# Toxicology Studies Needed for Licensure Biologics vs. Small Molecules

| Toxicity Testing | BLA Approval | NDA Approval |
|---|---|---|
| General Toxicity | Relevant species<br>(one species acceptable) | 2 species<br>(one rodent, one non-rodent) |
| Genotoxicity | Not required<br>(unless linker, contamination, direct interaction with DNA) | 3 assays |
| Safety Pharmacology | Often incorporated into general toxicology study | Often conducted with standard models |

# Toxicology Studies Needed for Licensure Biologics vs. Small Molecules

| Toxicity Testing | BLA Approval | NDA Approval |
|---|---|---|
| Reproductive Toxicity | -Relevant species<br>-Embryo-fetal development<br>-Select species closest to human physiology | Performed in at least 2 species, standard models of rat and rabbit |
| Carcinogenicity | -Interested in effects on cell proliferation, tumor promotion<br>-In vitro and in vivo studies (alternative models) | -Interested in direct interaction with DNA<br>-Performed in standard models (rodent), sometimes alternative models |
| Immunotoxicity | Interested in systemic exposure of biologic and consequence of anti-product antibodies | Centers on hypersensitivity and modification of immune system as an adverse event |

# Reproductive Toxicology Studies




ICHS5A and B: Reproductive Toxicology Guidelines

# Reproductive Toxicity
# Small Molecules

- Women of child-bearing potential may be included in early, carefully monitored clinical trials without reproductive toxicology studies provided appropriate precautions are taken to minimize risk
- Female fertility and embryo-fetal development studies should be completed before enrolling women of child-bearing potential in Phase 3 clinical trials

ICHM3: Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals

# Reproductive Toxicity Small Molecules

- The pre- and post-natal development and male fertility studies should be submitted with the licensing/marketing application
- All reproductive toxicity studies should be completed prior to any trial with pregnant women, children or women of childbearing potential not using highly effective birth control

# Reproductive Toxicology Biologics

- Studies defined in the ICH S5 and M3 documents are not the standard default
- Embryo-fetal toxicity studies are conducted for most compounds
- Need for fertility and pre- and post-natal development influenced by product, patient population and indication
- Timing of studies to clinical trials is flexible
- Limitations due to animal models (relevant species and immunogenicity)

# Carcinogenicity
# Biologics vs. Small Molecules

- Chronic administration (2 years) to rodents and assessment of tumor formation
  - Small Molecules
    - Performed for all products that are expected to be administered continually for 6 months to humans
    - 2 studies, mouse and rat
    - Due upon submission of NDA
  - Biologics
    - Typical carcinogenicity studies not usually performed for biologics, unless scientifically justified
    - Limitations: animal models and immunogenicity

# Carcinogenicity
# Biologics vs. Small Molecules

- Biologics may increase cellular proliferation and promote tumor growth
  - In vitro and in vivo studies to assess growth of tumor and normal cells
  - Evaluation of findings from long-term toxicology studies
  - Warning in the labels for immunosuppressants
  - Monitoring of patient populations

# Where Do I Go for Help?

## Guidance Documents

# Pharmacology/Toxicology Guidance Documents

- International
  - International Conference on Harmonisation (ICH) Documents

  http://www.ich.org/cache/compo/276-254-1.html
- CDER/CBER specific
  - Internally generated documents submitted for public comment

  http://www.fda.gov/cder/guidance/index.htm

  http://www.fda.gov/cber/guidelines.htm

# ICH Documents: Relevant to Biologics

- ICH S6: Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals
- ICH S5A &B: Detection of Toxicity to Reproduction for Medicinal Products
- ICH S7A: Safety Pharmacology Studies for Human Pharmaceuticals
- ICH M3: Nonclinical Safety Studies for the Conduct of Human Clinical Trials
- ICH Q5E: Comparability of Biotechnological/Biological Products Subject to Changes in their Manufacturing Process

# ICH Documents: Relevant for Small Molecules (selected)

- ICH S1A: Guideline on the Need for Long-Term Rodent Carcinogenicity Studies of Pharmaceuticals
- ICH S1B: Testing for Carcinogenicity of Pharmaceuticals
- ICH S1C(R): Guidance on Dose Selection for Carcinogenicity Studies of Pharmaceuticals: Addendum on a Limit Dose and Related Notes
- ICH S2A: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals
- ICH S2B: Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals
- ICH S5A: Detection of Toxicity to Reproduction for Medicinal Products
- ICH S7A: Safety Pharmacology Studies for Human Pharmaceuticals
- ICH M3: Nonclinical Safety Studies for the Conduct of Human Clinical Trials

# CBER/CDER Specific Documents

- Biologics
  - Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (CBER, 1997, undergoing revision)
- Small Molecules
  - Check CDER Web page

# Take Home Points

- Biologics and small molecules are inherently different
  - Microheterogeneity/ comparability
  - Relevant species
  - Immunogenicity
  - Clearance
- These differences are reflected in the nonclinical developmental strategies
- “One size fits all approach” not valid
- Guidances for nonclinical development can be found on CDER and CBER websites